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Short communication

Elevated carbon dioxide and irrigation effects on water stable aggregates in a *Sorghum* field: a possible role for arbuscular mycorrhizal fungi

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Abstract

While soil biota and processes are becoming increasingly appreciated as important parameters for consideration in global change studies, the fundamental characteristic of soil structure is a neglected area of research. In a sorghum [*Sorghum bicolor* (L.) Moench] field experiment in which CO_2 [supplied using free-air CO_2 enrichment (FACE) technology] was crossed factorially with an irrigation treatment, soil aggregate (1–2 mm) water stability increased in response to elevated CO_2. Aggregate water stability was increased by 40% and 20% in response to CO_2, at ample and limited water supply treatments, respectively. Soil hyphal lengths of arbuscular mycorrhizal fungi (AMF) increased strongly (with a threefold increase in the dry treatment) in response to CO_2, and the concentrations of one fraction (easily extractable glomalin, EEG) of the AMF-produced protein glomalin were also increased. Two fractions of glomalin, and AMF hyphal lengths were all positively correlated with soil aggregate water stability. The present results further support the hypothesis that AMF can become important in global change scenarios. Although in this field study a causal relationship between hyphal length, glomalin and aggregate stability cannot be demonstrated, the present data do suggest that AMF could mediate changes in soil structure under elevated CO_2. This could be of great importance in agricultural systems threatened by erosional soil loss.

Keywords: arbuscular mycorrhiza, elevated CO_2, FACE, global change, glomalin, soil structure, Sorghum

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Introduction

The empirical evidence supporting increases in atmospheric CO_2 (e.g. Keeling *et al*. 1995) and the number of studies describing the effects on ecosystems make rising atmospheric CO_2 concentrations one of the most significant factors of global change. A large proportion of CO_2 fixed in terrestrial ecosystems is allocated below ground, to roots (Rogers *et al*. 1994) and soil (e.g. Jones *et al*. 1998).

Besides serving as substrate for heterotrophic soil microbes that drive nutrient cycles, organic carbon in soil also plays an important role in soil aggregation (Kemper & Rosenau 1986; Degens 1997). Soil aggregates are groups of primary particles that adhere to each other

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more strongly than to surrounding soil particles (Martin *et al*. 1955). Soil structure and water-stable aggregation are crucial for facilitating water infiltration, soil-borne aspects of biogeochemical cycling processes, success of sustainable agriculture, and for providing resistance against erosional loss of soil (Oades 1984; Elliott & Coleman 1988; Van Veen & Kuikman 1990; Bethlenfalvay & Linderman 1992; Daily 1995; Arshad *et al*. 1996; Coleman 1996; Jastrow & Miller 1997; Young *et al*. 1998).

The consequences for soil aggregation of an elevated-CO_2-mediated increase in the allocation of carbon to soils has not been subject to intense study. Rillig *et al*. (1999a) provided evidence for a change in soil aggregate water stability in three natural ecosystems. Concurrently, an increase in hyphal length of arbuscular mycorrhizal fungi (AMF) was observed (Rillig *et al*. 1999b), and an increase in the soil concentrations of the protein glomalin was shown. Glomalin is a soil protein produced by AMF that is very tightly correlated with soil aggregate water stability (Wright & Upadhyaya 1998; Wright *et al*. 1999).

In this study it was hypothesized that under elevated CO_2 AMF extraradical hyphal length, glomalin concentration in soil, and soil aggregate water stability would be increased. This hypothesis was tested in a *Sorghum* field (equipped with a free-air CO_2 enrichment (FACE) system). During the last 40 years, nearly one third of the world's arable land was lost by erosion, with a current loss rate of more than 10 million hectares per year (Pimentel *et al*. 1995). Clearly, therefore, a CO_2-mediated increase of soil aggregate stability could be of particular importance in agroecosystems. In addition, it was tested whether the level of soil water supply could modify these responses to elevated CO_2, because the effects of soil water level (simulating either a management practice, or a global change factor) on AMF or glomalin concentration in soils are poorly understood.

Materials and methods

Design of the free air carbon enrichment (FACE) and irrigation field experiment

The free-air CO_2 enrichment (FACE) technique was used to enrich the air in circular plots within a 12-ha sorghum field (Trix clay loam soil; hyperthermic Typic Torrifluvents) at the University of Arizona Maricopa Agricultural Center (Maricopa, AZ, USA). The technique was similar to prior cotton and wheat experiments (e.g. Kimball *et al*. 1999). Briefly, four replicate 25-m-diameter toroidal plenum rings constructed from PVC pipe were placed in the field shortly after planting. Air enriched with CO_2 (200 μL L^{-1} above ambient) was blown into the rings. Carbon dioxide concentration was measured in the centre of each array at 10 cm above the crop canopy, and the mean daytime values were 566 μL L^{-1} and 373 μL L^{-1} for FACE and Control, respectively. The FACE treatment was applied continuously from emergence to plant maturity. Air blowers were installed in the non-CO_2-enriched ambient Control plots to provide air movement similar to that of the FACE plots.

Each of the main circular FACE and Control plots was split in semicircular halves, with each half receiving either an ample (wet) or a water-stress (dry) irrigation regime. Data were hence analysed as a strip-split-plot design. The water was applied using flood irrigation. As in similar previous experiments (Kimball *et al*. 1999), the criterion used to decide when to irrigate the Wet plots was after 30% of the available water in the rooted zone was depleted; they were then irrigated with an amount calculated to replace 100% of the potential evapotranspiration since the last irrigation. The total amounts of irrigation plus rain applied during 1998 were 1218 and 474 mm to the Wet and Dry plots, respectively.

Fertilizer was applied by air at a rate of 93 kgN ha^{-1} and 41 kgP ha^{-1}. The seed (*Sorghum bicolor* cv. Dekalb DK54) had been treated with fungicides, and was planted at a rate of 318 000 seeds ha^{-1}. There was only one mid-season irrigation for the dry plots, so a second application of fertilizer was applied to both the wet and dry plots on this date (11 September 1998) at a rate of 186 kgN ha^{-1} to give a total N application of 279 kgN ha^{-1} for the season.

Anthesis occurred on 1–2 October (wet) and 10–11 October (dry). Importantly, there was no difference in phenology (heading and anthesis dates) between FACE and Control treatments (P. Pinter, unpubl. obs.). The final grain harvest was carried out on 21 December 1998. One soil sample per treatment replicate was taken on 6 January 1999 to a depth of 30 cm (5 cm diameter), air-dried, and stored in plastic bags until analysis.

Glomalin

Glomalin extractions from soil were carried out as described by Wright & Upadhyaya (1998), where a more detailed discussion of glomalin can also be found. Easily extractable glomalin (EEG) was extracted with 20 mm citrate, pH 7.0 at 121 °C for 30 min. EEG is considered the most recently deposited fraction of the protein in soil (Wright & Upadhyaya 1998). Total glomalin (TG) was extracted with 50 mm citrate, pH 8.0 at 121 °C in rounds of 60 min each (two rounds were necessary). For the sequential extractions, the supernatant was removed by centrifugation at 5000 *g* for 20 min. Extraction of a sample continued until the supernatant showed none of the red-brown colour typical of glomalin. Extracts from each replicate were pooled and then analysed. After extraction cycles were completed, samples were centrifuged at

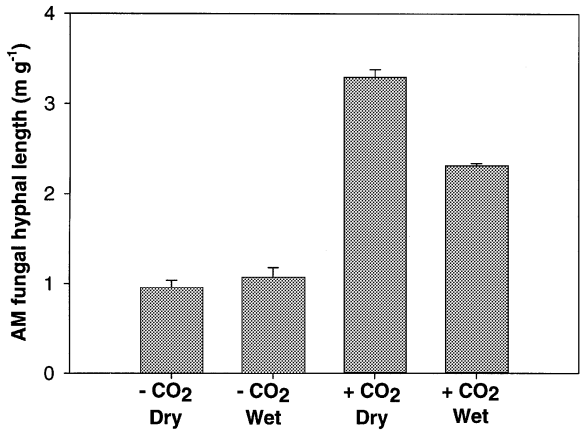

Fig. 1 Effects of elevated atmospheric CO_2 (free air carbon enrichment, FACE) and irrigation regime (Wet and Dry) on the length of soil hyphae of arbuscular mycorrhizal (AM) fungi. Error bars are standard errors of the mean ($n = 4$).

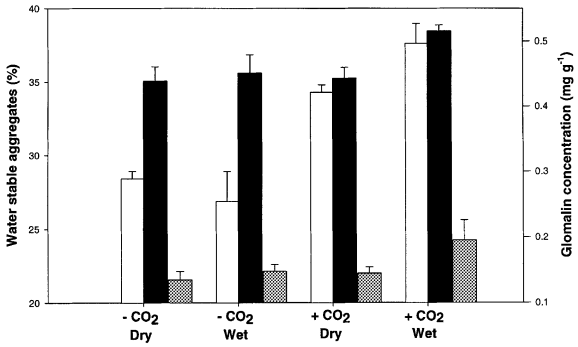

Fig. 2 Effects of elevated atmospheric CO_2 (free air carbon enrichment, FACE) and irrigation regime (Wet and Dry) on water stable aggregates (open bars), and soil glomalin concentrations (black bars, TG [total glomalin]; grey bars, EEG [easily extractable glomalin]). Error bars are standard errors of the mean ($n = 4$).

10,000 *g* to remove soil particles, and protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin as the standard (Wright & Upadhyaya 1998). Concentration of glomalin was extrapolated to mg g^{-1} by correcting for the dry weight of coarse fragments included in the extraction of soil.

Arbuscular mycorrhizal fungal hyphae and water-stable aggregates

Hyphae were extracted from a 4-g soil subsample by an aqueous extraction and membrane filter technique modified after Jakobsen *et al*. (1992), as described in Rillig *et al*. (1999b). All soils had been stored as air-dried samples > 4 months prior to aggregate analysis. Macro-aggregates of 1–2 mm diameter were used primarily, because the amounts of these aggregates are sensitive to short-term (< 2 years) management and treatment of soils (Kemper & Rosenau 1986). Replicate 4 g samples of soil were moistened by capillary action for 10 min. Water-stability of aggregates was then measured with a wet-sieving method using the apparatus and procedure described in Kemper & Rosenau (1986). The initial and final weights of aggregates were corrected for the weight of coarse particles (> 0.25 mm). Aggregate stability is the mass of aggregated soil remaining after wet sieving as a percent of the total mass of soil.

Results and discussion

Arbuscular mycorrhizal hyphal lengths in soil (Fig. 1) were clearly increased in response to elevated CO_2 ($F_{CO2} = 456.6$; $P = 0.0002$). There was no significant effect of irrigation regime at the control level of CO_2, but under FACE, hyphal lengths were significantly increased under dry compared to wet irrigation level. ($F_{Water} = 50.37$; $P = 0.0004$; $F_{Water \times CO2} = 80.37$; $P = 0.0001$). It is speculated herein that under dry irrigation level, soil nutrients may have been less mobile (and hence, mass flow to the roots smaller). With assumed higher plant nutrient demand under elevated CO_2, these are conditions that could favour increased plant dependence on AMF delivery of nutrients (Smith & Read 1997), and hence production of soil hyphae. Increased AMF hyphal lengths under elevated CO_2 have been documented in previous pot experiments and in field situations, but rarely under conditions of agricultural cultivation (e.g. Klironomos *et al*. 1997; Sanders *et al*. 1998; Rillig *et al*. 1999b). It is worth noting that more than a threefold increase in hyphal length was found under elevated CO_2, which is highly disproportionate to typical plant growth responses to CO_2. Because the relationship between plant/root and AMF hyphal growth is poorly understood, a mechanistic explanation for this result cannot be proposed. Physiologically and architecturally, fungi and plants are clearly quite distinct. For example, growth in fungi occurs as tip growth for hyphae, in contrast to meristematic growth for plants. Therefore, there is no *a priori* reason to expect the fungus and the plant to show the same proportional response to any given global change factor.

The percentage of water stable aggregates (1–2 mm; Fig. 2) in these soils was increased significantly ($F_{CO2} = 43.37$; $P = 0.0001$) by the CO_2 treatment, but it did not respond to water treatment ($F_{Water} = 0.50$; $P = 0.49$). There was a weak, but not significant, interaction between water and CO_2 treatment ($F_{Water \times CO2} = 3.69$; $P = 0.08$). The CO_2 portion of the results is in general agreement with the present authors' previous finding of

increased water-stability of aggregates under elevated CO_2 (Rillig *et al*. 1999a). Importantly, the increase resulting from CO_2 observed in this study is much higher than previously measured: water stability here increased by approximately 40% in the wet treatment and by approximately 20% in the dry treatment in response to CO_2. It is thus demonstrated for the first time that elevated CO_2 can affect soil aggregation in an agricultural system. In agricultural systems, a soil stabilizing effect of CO_2 would be clearly advantageous (Pimentel *et al*. 1995). More studies are needed, however, to show that this increase in stable soil aggregates in response to CO_2 can be generalized to a variety of agricultural cropping systems. Extrapolations from this study alone are problematic, with sample size being (by necessity) relatively low ($n = 4$).

One of the goals of the present study was to assess whether glomalin concentrations (TG or EEG) would also respond to CO_2 and could be used as indicators of changes in water stability of aggregates. TG was not significantly changed in response to CO_2 ($F_{CO2} = 2.42$; $P = 0.19$), but EEG was ($F_{CO2} = 10.41$; $P = 0.02$). While EEG was decreased by drought ($F_{Water} = 5.08$; $P = 0.06$), there was only a trend for decrease in TG in response to drought ($F_{Water} = 4.00$; $P = 0.11$). There was a trend for a treatment interaction for TG ($F_{Water \times CO2} = 5.40$; $P = 0.11$) and EEG ($F_{Water \times CO2} = 4.36$; $P = 0.08$). It thus seems that although glomalin concentrations were changed in response to CO_2, there was no close relationship between the treatment responses of water-stable aggregates and glomalin. Therefore, it was tested whether glomalin concentrations, and also hyphal lengths, across all treatments were correlated with water stability of aggregates. Although the percentage of water-stable aggregates was correlated (Fig. 3) with TG, EEG and AMF hyphal length, AMF hyphal length and TG ($r^2 = 0.009$; $P = 0.71$) and AMF hyphal length and EEG ($r^2 = 0.03$; $P = 0.51$) were not correlated in this study. This is not very surprising, because hyphae and glomalin may have completely different turnover times (Rillig *et al*. unpubl. data), and hence differences in decomposition rates of hyphae and glomalin could alone account for a lack of correlation. Additionally, treatments could have caused a shift in AMF species composition; this could have lead to altered production of glomalin if AMF differ in glomalin yield per hyphal length (Wright *et al*. 1996).

Soil aggregation is a very complex hierarchical process (e.g. Tisdall & Oades 1982); however, the concentration of glomalin in 1–2 mm aggregates is very tightly correlated with the water stability of these aggregates across different soils (Wright & Upadhyaya 1998). It has been shown in the present study (Fig. 3) that within a single soil exposed to different global change treatments total soil concentration of glomalin (as opposed to

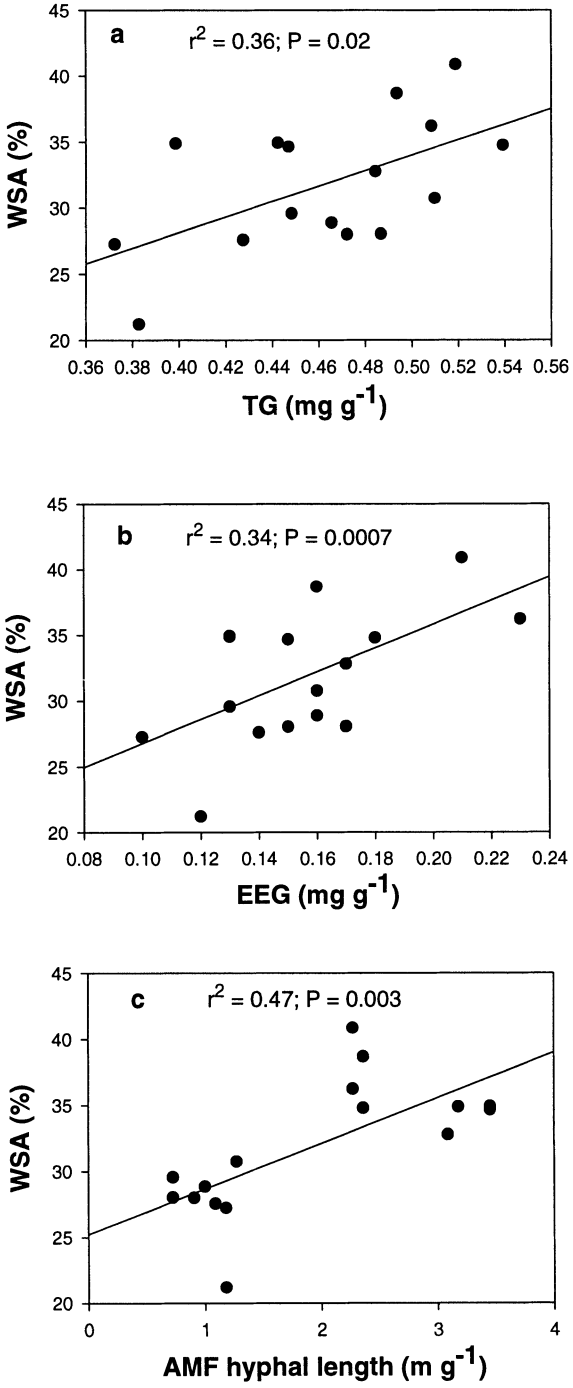

Fig. 3 Relationship between percentage of water-stable aggregates (WSA%) and TG (a), EEG (b), and AM fungal hyphal length (c).

glomalin extracted from aggregates) was correlated (albeit less strongly) with aggregate water stability of 1–2 mm aggregates. However, glomalin concentrations (TG or EEG) did not always closely track changes in soil aggregation for the four treatment combinations. In these cases, other soil factors could have been more important

than glomalin in determining water-stability, illustrating our current lack of understanding of the mode of action of this protein. Alternatively, small samples sizes could have contributed to this response. Nevertheless, the present results suggest that soil aggregate water stability must be considered in global change studies, and that the usefulness of glomalin as an indicator of altered aggregate stability should be further explored.

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